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Validation of yield-enhancing quantitative trait loci from a low-yielding wild ancestor of rice

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Abstract A set of introgression lines (ILs) containing chromosomal segments from *O. rufipogon* (IRGC 105491), a wild relative of *O. sativa*, in the genetic background of an elite US variety, cv. Jefferson, was developed to confirm the performance of six yield-enhancing quantitative trait loci (QTL). Fifty BC3F3 ILs containing homozygous *O. rufipogon* introgressions at each of the target QTL regions, and as few background introgressions as possible, were selected for evaluation of yield and 14 yield-related traits in field studies conducted over 2 years at four locations in the southern USA. Performance of the IL families was compared with three commercial inbreds and one hybrid variety. IL families carrying introgressions from the low-yielding wild parent at the QTL *yld2.1* and *yld6.1* yielded 27.7 and 26.1 % more than Jefferson, respectively. IL yld2A, which possesses *yld2.1*, also performed well under alternate wetting and drying conditions in two field locations. After the first year of field trials, 10 of the top-performing BC3F4 families, representing five of the QTL targets, were genotyped with an Illumina 1,536 assay to define the size and location of the wild introgressions. BC3F4 families with the fewest background introgressions were backcrossed to Jefferson and selfed. The resulting BC4F2 families were screened with targeted single nucleotide polymorphism assays to identify individuals carrying homozygous introgressions across the target QTL. Twelve ILs, representing each of the six QTL targets, have been submitted to the Genetic Stocks *Oryza*

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Collection for studies on transgressive variation and as interspecific pre-breeding lines.

Keywords Near isogenic lines · Introgression lines · Crop yield · Quantitative trait loci · Genetic analysis · Genotypes

Introduction

The development of near isogenic lines (NILs) that incorporate introgressions from genetically divergent donors in the background of elite, high-yielding cultivars is an effective strategy for evaluating the genetic potential of wild and exotic alleles at candidate quantitative trait loci (QTL). This strategy is based on first identifying desirable QTL in a segregating population derived from a bi-parental cross. Once desirable QTL are detected, introgression lines (IL) or NILs carrying a single target QTL can be developed through backcrossing and marker-assisted selection (MAS) (Eshed and Zamir 1995; Yano and Sasaki 1997; Young and Tanksley 1989). The performance of NILs can be used to evaluate the effect of target QTL, and NILs can be sib-mated to construct desired combinations of introgressions in the background of an elite cultivar. This strategy has been used as the basis for introgressing exotic alleles into adapted breeding lines of wheat, barley, soybean, tomato (Krystkowiak et al. 2009; Schmalenbach et al. 2009; Stupar 2010; Chapman et al. 2012) and rice (McCouch et al. 2007; Fukuoka et al. 2010).

In rice, QTL introgressed from exotic landraces and/or wild species have conferred large and highly significant effects on yield under both irrigated and, more recently, water-limited conditions (Moncada et al. 2001; Bernier et al. 2007, 2008, 2009a, b; Uga et al. 2011; Venuprasad et al. 2009, 2011, 2012; Kumar et al. 2009). Under lowland drought stress, ILs out-yielded the susceptible recurrent parents by ~44 % and under upland drought conditions by ~93 % (Venuprasad et al. 2011). Recently, cloning of the rice gene, *DEEPER ROOTING 1 (DRO1)*, underlying a highly significant QTL demonstrated that one or a few genes can have a dramatic effect on yield under stress (Uga 2012). Consistent with these reports, a recent study in tomato reported a 50 % yield increase based on pyramiding three independent introgressions from a wild tomato in the genetic background of a leading market variety (Gur et al. 2011).

In rice, the relative ease of identifying and incorporating alleles with large effect through marker-assisted backcrossing makes this method a viable approach to rice improvement (Ashikari et al. 2005; Collard and Mackill 2008; Thomson et al. 2011). In contrast, introgression of yield-related QTL into elite backgrounds of wheat showed only moderate gains (Miedaner et al. 2009; Kumar et al. 2010, 2011), and in maize no major-effect QTL have been reported for yield or any yield-related trait. In maize and other outcrossing species, complex traits such as yield, disease resistance and flowering time are typically controlled by many small-effect QTL with an additive effect (Buckler et al. 2009; Poland et al. 2011), requiring a very different approach to genomic-assisted plant improvement.

In a previous study from our laboratory, Jefferson, a US *tropical japonica* rice cultivar, was crossed with its wild progenitor species, *Oryza rufipogon*, and QTL analysis was conducted on the BC2F2 generation evaluated in four field environments in Arkansas and Texas (Thomson et al. 2003). In the current study, ILs carrying six yield QTL identified by Thomson et al. (2003) were developed and evaluated in multi-location yield trials in the southern USA. The best performing BC3F3 IL families were selected for further backcrossing using MAS. The subsequent BC4 families retain the target QTL, but have reduced number and size of background introgressions. A yield QTL in the same region on chromosome 2 as identified by Thomson et al. (2003) has been shown to contribute to increased yield in hybrids grown in China (McCouch et al. 2007; Xiao et al. 1998; Wu et al. 2010).

In the present study, we (a) developed ILs in the Jefferson background for six yield QTL through marker-assisted backcross selection, (b) tested the performance of the ILs over 5 years in replicated field trials at four locations in the southern USA, (c) submitted ILs representing each target QTL to the Genetic Stocks *Oryza* (GSOR) Collection (http://www.ars.usda.gov/Main/docs.htm?docid=14200), (d) released the top-performing IL for use as a parent in rice breeding programs, and (e) identified single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers to accelerate the introgression of these

O. rufipogon-derived QTL into new genetic backgrounds.

Materials and methods

Plant material

The recurrent parent, Jefferson (*Oryza sativa* L. *tropical japonica*) (Reg. no. CV-103, PI593892, GS*OR*303013), is an elite, long-grain, blast disease-resistant, semi-dwarf variety developed for the US market (McClung et al. 1997) that was released in 1998. The donor parent is an *aus*-like accession of *O. rufipogon* (IRGC105491, GS*OR*303014) from Malaysia. It has no agronomic traits of interest but was used as the donor because it crosses readily with diverse *O. sativa* varieties (i.e. *indica* and *japonica*) (McCouch et al. 2007).

Fourteen BC3F2 families carrying a favorable introgression from *O. rufipogon* (IRGC105491) at one of the six yield-related QTL identified by Thomson et al. (2003) were selected as the starting point for this study (Table 1). Fifty ILs (4–12 BC3 families per target QTL) were developed, along with 20 control sib-lines (2–5 BC3 families per QTL that lacked the target introgression). Henceforth we will refer to QTL in italics and without prefix or suffix (e.g., *yld2.1*), while we will refer to lines containing the QTL using the prefix 'IL' and no italics (e.g., IL yld2_A).

Within families, individuals homozygous for the introgressions of interest were backcrossed to Jefferson and self-pollinated (Supplemental Fig. 1). During the course of IL development, progeny were eliminated that were highly sterile, extremely late, had dormancy, shattering, and/or red bran (Thomson et al. 2003).

The performance of BC3 introgression lines (ILs) and BC4 IL derivatives was compared with that of Jefferson and four commercial checks, Cocodrie (PI606331), Trenasse (PI641796), Presidio (PI636465) and the hybrid cultivar XL723 (RiceTec, Inc., Alvin, TX, USA) in field trials in Arkansas and Texas during 2007–2010.

Backcrossing and marker-assisted selection procedure

For both the BC3 and BC4 populations, Jefferson, the recurrent parent, was used as the female during backcrossing and selected ILs were used as pollen parents, except for family 85 (targeting the yield QTL, *yld2.1*) where asynchrony of flowering required that Jefferson be used as the male. Markers across the target QTL regions were used to screen BC populations each generation to eliminate the 50 % of plants

Table 1 Six target QTL with corresponding peak marker from Thomson et al. (2003)

QTL	QTL reported by Thomson et al.	BC2F2 family ID	Chr	Peak marker	Position (Mb) MSU v.6[b]	Flanking markers position (Mb)[c]	LOD score	R^2
yld1.1	*gpp1.2*[a]	89; 158; 185	1	RM5[a]	*23.9715*	23.9713–30.0275	6.35[a]	9.7[a]
yld2.1	*gpp2.1; spp2.1; yld2.1*	43; 141; 85	2	CDO718	*18.5343*	18.5342–18.5345	7.35	12.3
yld3.2	*gpp3.1; spp3.1; pss3.1; dth3.4; gw3.2; yld3.2*	16	3	RG1356	*35.1410*	33.3863–35.1411	11.56	16.6
yld6.1	*pss6.1; yld6.1*	219; 221	6	RM276	*6.2301*	6.2300–6.2302	3.74	6.5
yld8.1	*gpp8.1; ph8.1*	307; 338	8	RM210	*22.4719*	22.4718–22.4720	4.44	7.2
yld9.1	*pl9.1; tt9.1; yld9.1; gpp9.1; spp9.1*	9; 13; 16	9	RM215	*21.1892*	21.1891–21.1893	4.28	7.4

yld yield, *gpp* grains per panicle (average number of filled spikelets per primary panicle), *spp* spikelets per panicle (average total number of spikelets per primary panicle), *pss* percent seed set (gpp divided by spp), *dth* days to heading, *gw* grain weight, *ph* plant height, *pl* panicle length, *tt* tiller type (erect or lazy tillering), *RM* rice microsatellite, *CDO* cDNA from oat used as RFLP marker, *RG* rice genomic RFLP marker

[a] These QTLs and associated scores were detected in the BC2F2 generation (Thomson et al. 2003). While the current study defines yield as kilograms per hectare, the original yield measurement were determined by the average weight of bulked harvested grain per plant from at least ten plants

[b] Rice pseudo-molecules assembled by Michigan State University Version 6 http://rice.plantbiology.msu.edu/

[c] Marker IDs can be found in paper by Thomson et al. (2003)

that did not carry the *O. rufipogon* allele. Selected plants with the target introgression at the QTL were then screened for other introgressions to identify lines carrying the least amount of *O. rufipogon* DNA in non-target regions.

Markers and marker assays

DNA was extracted from single plants using one of the following methods: (a) Qiagen DNeasy Plant Tissue Kit following protocols provided by Qiagen Inc. (Valencia, CA, USA), (b) CTAB method as described by Fulton et al. (1995), or (c) Extract-N-Amp Plant Kit (Sigma-Aldrich, Saint Louis, MO, USA).

The BC3 material was genotyped with SSRs (IRSP 2005; www.gramene.org) and two fixed SNP arrays: (1) a custom-designed 1,536-SNP Illumina GoldenGate assay (Zhao et al. 2010) and (2) a custom-designed 384-SNP Illumina BeadXpress assay (OPA6.0, described in Thomson et al. (2011). BC4 families were genotyped with insertion/deletion (InDel) and targeted SNP markers using competitive allele-specific PCR KASP chemistry (KBiosciences Ltd, Hertfordshire, UK) and MassARRAY iPLEX® Gold (Sequenom, Inc., San Diego, CA, USA) assays. SNP positions targeted for KASP genotyping were identified based on the custom-designed 44K-SNP Affymetrix rice chip (Zhao et al. 2011), which provided sufficient density of SNPs so that informative markers could be designed every megabase across the target regions of interest. Design of primers for KASP genotyping (three primers per SNP) was accomplished using the online tool, 'PrimerPicker', and subsequently using 'KASP by design'. The list of primers used for KASP marker detection is provided in Supplemental Table 1.

Additional genotyping was conducted with MassARRAY iPLEX® Gold. Three multiplexes (26–28 SNPs per assay) were designed using SNP targets selected from the 44K chip (Zhao et al. 2011) at 0.5-Mb intervals across the target regions (Supplemental Table 2), and including many SNPs that overlapped with the KASP assay. The protocol is outlined in the application notes found online (Oeth et al. 2005; http://www.sequenom.com/sites/genetic-analysis/applications/snp-genotyping).

Different DNA extraction methods were used with different marker assays. For use with genome-wide SNP assays, including the 44K-SNP array (Zhao et al. 2011), the 1,536-SNP OPA (Zhao et al. 2010) and the 384-SNP OPA (VC0011530-OPA) (Thomson et al. 2011), we used DNA samples extracted using Qiagen DNeasy Kits. For KASP assays, the Extract-N-Amp procedure was used with an additional overnight dry-down step with 2 µl of extracted DNA diluted in 98 µl of ddH_20 placed in 384-well PCR plates (D. Wang, Cornell University, personal communication). For MassARRAY iPLEX® Gold assays, CTAB extractions included additional treatments of RNase (1 µl of 10 mg/ml RNase with 100 µl of TE buffer for 1 h in 37 °C bath after resuspending DNA pellet) and dissolving DNA in 50 µl of AE buffer (Qiagen) to enhance the quality and reliability of the assays. We also tested the MassARRAY SNP genotyping platform using three sets of DNA samples: Qiagen Plant Mini Kit-extracted DNA (set 1), chloroform-extracted DNA (set 2), and various concentrations of lesser quality Extract-N-Amp DNA samples (set 3–5), and found that the Qiagen Plant Mini Kit extractions gave the best performance.

SSR markers used to detect the presence/absence of target introgressions were selected from the pool of SSRs reported for rice (IRGSP 2005) and available on the Gramene database (http://www.gramene.org/). The sizes of SSR fragments were estimated using the software GeneMapper v 3.7 (Applied Biosystems, Foster City, CA, USA). InDel markers were identified based on a comparison of the Nipponbare and 9,311 sequences across the target regions (http://www.ncbi.nlm.nih.gov/). A list of primers used for InDel marker detection is given in Supplemental Table 3.

Information on markers associated with major genes for rice blast resistance and for the grain quality traits amylose content, gelatinization temperature and aroma are presented in the Supplemental Note on Gene-Specific Markers.

Phenotyping of yield and yield components

During 2007–2008, 50 BC3 IL families and 20 control sib-families were evaluated in flooded paddies at four locations: Stuttgart (AR), Jonesboro (AR), Beaumont (TX), and Alvin (TX). In 2008, eight NILs were removed because they had red bran. The experiments were conducted using a randomized complete block (RCB) design with three replications. Each plot was approximately 5.8 m^2 and was drill-seeded at approximately 45 kg ha^{-1}, a low seeding rate commonly

used for hybrids. Prior to planting, 112 kg ha^{-1} of fertilizer nitrogen was applied and incorporated. Plots were flush-irrigated until stand establishment and then maintained under a permanent flood. Common pesticides were used to manage weeds throughout the season.

During 2008, the BC3 ILs were also evaluated at Stuttgart (AR) and Beaumont (TX) under alternate wetting and drying (AWD) conditions. The same procedures were used as in the flooded plots except that, after irrigation to achieve stand establishment, the plots were subsequently irrigated only after the ground had dried to the point of cracking.

Plots were evaluated for days to heading, plant height and lodging percentage. Plots were harvested at approximately 18–20 % grain moisture with a combine harvester to determine grain yield (in kg ha^{-1} adjusted to 12 % moisture). At early tillering, two plants in each plot were identified for use in yield component measurement and were hand-harvested prior to plot yield determination.

Yield components included average panicle length (AVPANL), average seeds per panicle (AVSDPAN), average panicle weight (AVPANWT), average tiller number (AVTILL), average plant weight (AVPLTWT) and 1,000-seed weight (KSDWT). Days to heading (D2HD), plant height (PLTHT), lodging percent (Lodge), plants per square meter (PLSQM) and percent stand (STDPCT) were measured. Cleaned rough rice samples (125 g) were milled using a McGill No. 2 mill (Rapsilver Supply Co Inc., Brookshire, TX, USA) according to a standard protocol for long-grain rice. Total milled rice percentage (TotalMY) was determined using the weight of the whole plus broken kernels as a proportion of the rough rice sample. The milled rice was separated using a #12 screen (Seedburo Equipment Co., Chicago, IL, USA) and the weight was used to determine the whole milling yield (WholeMY) as a percent of the rough rice. Grain length (GL), grain width (GW) and percent chalk (CHKPCT) were determined using a WinSeedle Pro 2005a^{TM} image analysis system (Nelson et al. 2011). Apparent amylose content (AMYLOSE) was determined using the modified iodine spectrophotometric method of Perez and Juliano (1978) with a continuous-flow analyzer (AutoAnalyzer 3 Seal Analytical, Mequon, WI, USA). Alkali spreading value (ASV), an indicator of starch gelatinization temperature, was determined on six milled kernels using sodium hydroxide digestion according to the methods of Little et al. (1958).

During 2009, two selected BC3 ILs were evaluated in six locations as part of the Uniform Regional Rice Nursery (URRN): Crowley (LA), Stuttgart (AR), Malden (MO), Stoneville (MS), Beaumont (TX), and Eagle Lake (TX). Among a set of breeding lines evaluated in the URRN were two of the cultivars that were used in the 2007–2008 field trials, Cocodrie (released in 2000) and Trenasse (released in 2006), along with Presidio, a 2008 release that is a derivative of Jefferson. The study was conducted as a RCB design with two to four replications depending on the location. Yield plots were approximately 5.8 m^2 and were drill-seeded using a 125 kg ha^{-1} seeding rate. Fertilizer and pesticides were used according to local recommendations to maximize yield potential. Least squares means of grain yield, total and whole milling yield were evaluated in the URRN trials.

During 2010 and 2011, BC4 ILs developed from the two selected families representing *yld2.1* and *yld6.1* were evaluated in replicated field trials conducted in Stuttgart (AR) (2010 and 2011) and Beaumont (TX) (2011). The studies were conducted using a RCB design with three replications and standard yield plots, as described previously. The entries included 18 BC4 ILs derived from the *yld2.1* and *yld6.1* BC3 generation, five of the original BC3 representatives, and the commercial check cultivars, including Jefferson, Trenasse, Cocodrie and Presidio. Cultural management practices for optimizing yield at each of the locations were used, as described previously.

Statistical analysis

In the irrigated system all variables were analyzed using the MIXED Procedure in SAS version 9.2 (copyright 2002–2010 by SAS Institute Inc., Cary, NC, USA). The fixed effects included Line (i.e., Genotype), Location, and their interaction, while Block, Year, and their interaction were treated as random effects. The AWD analysis did not include a year effect. Least-squares (LS) means and differences of the Line means compared to Jefferson and other controls were calculated using the LSMEANS option and diff test with Dunnett's adjustment. Multiple Line to Line comparisons were achieved with the pdiff test option and family-wise error rate was controlled with the Tukey–Kramer adjustment for

p values. Regression analyses were performed for each introgression family using the REG Procedure in SAS 9.2. The forward selection method was employed to fit the independent variables (traits) using a criterion of *p* value less than 0.25. Pearson correlation calculations were achieved with the CORR procedure in SAS 9.2. In assessing genotype × environment (G × E) interactions, both the irrigated plots and AWD plots from 2008 in Beaumont and Stuttgart were combined for analysis. The MIXED procedure in SAS 9.2 was utilized, with Location and Irrigation method, and their interaction defined as fixed effects, and Block effect and its interactions as the random effects.

Huhn's nonparametric stability statistics, based on ranks of Lines in each environment, were estimated using the MEANS procedure and RANK procedure in SAS 9.2, similar to SAS coding statements available in Lu (1995).

Data from the 2009 URRN trial conducted at six locations was analyzed using the MIXED procedure (SAS 9.2) with State and Line, and their interaction considered as fixed effects, and the Replication and its interaction with State and Line as random effects. Significant covariate effects were found for yield, height and days to heading with whole and total milling yields. These covariates were used to determine adjusted least squares means.

Results

Multi-location field trials (2007–2008)

Fifty BC3 ILs carrying six different yield QTL from *O. rufipogon* (on chromosomes 1, 2, 3, 6, 8 and 9) were developed in the Jefferson background over the course of this project and evaluated over 2 years (2007–2008) in multi-location yield trials in the southern USA to confirm the impact of the *O. rufipogon* QTL alleles on yield of the ILs (Supplemental Fig. 2; Table 2). ILs carrying introgressions across *yld2.1* and *yld6.1* (previously reported by Thomson et al. (2003)) consistently out-performed the recurrent parent, Jefferson, in grain yield per plot (Table 2). The other four ILs, with introgressions on chromosomes 1, 3, 8 and 9, significantly out-performed Jefferson in some environments but not others. Eight of the lines that performed best in the 2007–2008 trials have been selected for submission to the Genetics Stocks *Oryza* Collection for use as pre-breeding materials (Table 3).

All 50 of the BC3 ILs carried introgressions across the target QTL regions, and in addition, random background introgressions on other chromosomes that were identified using SNP panels (Fig. 1; Table 3). The best line, IL yld2_A (family 43_1-2), yielded 27.7 % better than Jefferson based on average grain yield across four locations during 2007–2008 (Table 3). This line contained an 11.7-Mb target introgression on chromosome 2 plus four background introgressions (Fig. 1b). The second-best line, IL yld6_A (family 219_2-9), contained a 14.2-Mb introgression on chromosome 6 plus two small background introgressions (Fig. 1d), and out-yielded Jefferson by 26.1 % (Table 3).

To determine the effect of the spurious *O. rufipogon* introgressions in the genetic background of these families, we compared the performance of IL yld2_A with that of a sister line, IL yld2_B (family 43_2-12). These sib-ILs were genetically identical across the target region, but IL yld2_B lacked two of the background introgressions (a 9.2-Mb introgression on chromosome 5 and a 1.5-Mb introgression on chromosome 9) that were present in IL yld2_A (Fig. 1b). IL yld2_B out-yielded the recurrent parent, Jefferson, by 21.2 % in the multi-location trials (Table 3), but it yielded less (though not significantly) than IL yld2_A. A similar trend was observed when sib-lines IL yld6_A and IL yld6_B were compared: the lines were identical across the target region on chromosome 6, but IL yld6_B contained a smaller introgression on chromosome 11 than IL yld6_A. The loss of ~3.5 Mb of *O. rufipogon* DNA corresponded to a slight reduction in yield compared to IL yld6_A, though IL yld6_B still yielded significantly better than Jefferson (24.9 %). Thus, we conclude that the additional background introgressions in these ILs do not negatively affect yield performance, and may even enhance it.

To better evaluate the impact of the target QTL introgressions on yield performance, 20 'control' lines were selected from within each of the segregating BC3 families. These control lines retained the random assortment of *O. rufipogon* background introgressions found in each family, but lacked the *O. rufipogon* introgression at the peak marker for each QTL (Table 2). This provided a way of testing the effect of only the target introgression for each yield QTL.

Table 2 Yield of 50 ILs encompassing six yield-QTL targets and 20 control sib-lines evaluated over eight flooded field environments

	yld1.1	Lsm	*yld2.1*	Lsm	*yld3.2*	Lsm	*yld6.1*	Lsm	*yld8.1*	Lsm	*yld9.1*	Lsm
Lines tested												
1	158_1-5	6,919.4[d]	**43_1-2***	**7,937.7**	16_1-4	7,151.1	219_1-5*	7,762.6	307_1-5	6,778.1	9_2-9	6,275.0
2	158_1-7	7,014.8	43_1-4*	7,800.3	16_1-12	7,044.5	**219_2-9***	**7,838.6**	307_1-6	6,885.5	9_2-10[a]	6,363.9
3	158_2-6	7,146.9	43_1-5*	7,645.0	16_2-1	7,306.2	221_1-11	6,863.8	307_1-12	6,913.4	13_1-1	7,176.3
4	158_2-7	7,428.0	43_2-1*	7,543.1	16_2-11	6,745.4	221_2-4	6,401.1	338_1-3	6,749.1	13_1-3	6,878.6
5	158_2-12[a]	7,178.9	43_2-8*	7,779.0					338_1-4	7,053.1	13_2-9	6,941.0
6	185_2-4	6,747.3	43_2-12*	7,534.9					338_2-11	7,299.6	13_2-10	7,153.6
7	185_2-11	6,857.6	141_1-1	6,963.0					121_2-2[b]	7,242.9	16_1-1	6,388.6
8	89_1-5	6,684.3	141_2-3	6,927.9					121_2-8[b]	7,169.4	16_1-2	6,990.4
9	89_1-7	7,066.5	141_2-6	6,752.2					121_2-12[b]	7,239.9	16_1-10	6,813.8
10	89_2-5	6,278.9	141_2-11	7,175.2							13_1-12[b]	6,014.3
11	89_2-6	6,841.7									13_2-2[b]	6,752.5
12											13_2-5[b]	6,119.1
Controls												
1	185_2-2	6,571.3	141_1-8	5,836.0	16_1-6[c]	6,025.8	219_1-12	6,818.2	338_2-3	6,559.3	9_1-3	7,033.0
2	185_2-3[a]	6,195.2	141_2-1	6,390.5	16_2-3[c]	6,499.4	219_2-10	6,681.4	338_2-5	6,587.1	9_1-7	6,947.3
3	185_2-6	6,384.0	141_2-9	6,129.4			219_2-11[a]	6,596.7	121_1-1[b]	6,656.7	9_2-2	6,804.4
4							221_1-4	6,036.2	121_2-3[b]	7,027.5	16_1-6[c]	6,025.8
5							221_1-13	6,642.8			16_2-3[c]	6,499.4
		Recurrent parent, Jefferson										
		Lsm	± Stderr									
		6,216.8	809.7									

The best performing lines entered into the URRN (Uniform Regional Rice Nursery) trials are indicated in bold; *Lsm* least square mean of yield (kg ha^{-1}) under flooded field conditions; degrees-of-freedom = 65

*indicates the highest performing families carry *yld2.1* and *yld6.1*

[a] Stderr = 824.6 for lines 158_2-12, 219_2-11, and 9_2-10; stderr = 831.6 for line 185_2-3

[b] These eight lines were omitted from the 2008 field trials due to red pericarp or segregation of red pericarp evident in the previous year. Lsmeans standard error was 861.5 for families 121 and 13 except for 121_1-1 with a standard error of 865.4

[c] Note that values for control lines 16_1-6 and 16_2-3 are duplicated to represent control lines for both *yld3.2* and *yld9.1*; these lines are also of interest as donors of sheath blight resistance, and because line 16_1-6 had significantly lower levels of chalk than other lines

[d] Standard error (stderr) values for all ILs that were tested in both 2007 and 2008 were 823.8 with exception of lines denoted with [a]

Table 3 Yield performance of lines submitted to the Genetic Stocks *Oryza* (GSOR) Collection under flooded conditions in four locations during 2007–2008

Line ID	Line name	GSOR ID	Target QTL	% Donor genome	Target introg. size (kb)	# Bkg introg.	2007–2008 Lsmean yield	Lsmean stderr	2007–2008 % diff to Jeff
yld1_A	158_2-7	303001	1.1	3.1	4,158	0	7,428.0	823.8	19.4
yld2_A	43_1-2*	303002	2.1	6.7	11,714	4	7,937.7	823.8	27.7*
yld2_B	43_2-12*	303003	2.1	5.6	11,714	3	7,534.9	823.8	21.2*
yld2_C	43_1-2_7-1[a]	303004	2.1	7.8	11,714	3	–	–	–
yld2_D	85_2-8_16-8	303005	2.1	9.7	9,920	2	–	–	–
yld3_A	16_2-1	303006	3.2	11.94	6,373	5	7,306.2	823.8	17.5
yld3_B	16_2-1_17-3[a]	303007	3.2	4.9	7,001	2	–	–	–
yld6_A	219_2-9*	303008	6.1	5.6	14,160	2	7,838.6	823.8	26.1*
yld6_B	219_1-5*	303009	6.1	4.9	14,160	2	7,762.6	823.8	24.9*
yld6_C	219_1-5_29-7[a]	303010	6.1	5.6	14,160	0	–	–	–
yld8_A	121_2-2	303011	8.1	16.38	8,280	6	7,242.9	861.5	16.5
yld9_A	13_1-1	303012	9.1	2.9	5,295	2	7,176.3	823.8	15.4
	Jefferson						6,216.8	809.7	NA

GSOR ID Genetic Stock *Oryza* identification number, *Target QTL* QTL as defined in Table 1, *% Donor genome* percentage of donor genome calculated by the number of polymorphic markers out of total markers genotyped *% Donor genome* for BC3 and BC4 generation ILs was calculated based on different SNP genotyping platforms, which accounts for apparent inconsistency, *Target introg. size (kb)* size of the introgression at the target QTL, *# Bkg introg.* number of *O. rufipogon* introgressions in genetic background, *2007–2008 Lsmean yield* least squares mean yield for 2007–2008 across four flooded locations, *Lsmean stderr* standard error of the mean, *2007–2008 % diff to Jeff* yield performance in 2007–2008 of lines compared to the recurrent parent, Jefferson, calculated as the percent improvement

*indicates accessions with the greatest yield improvement as compared to Jefferson

[a] Derived BC4 lines which were not available for the 2007–2008 field season

The yield performance of the controls was consistently lower than that of the corresponding ILs that carried the target introgression, with one exception in the case of *yld9.1* (Fig. 2). This confirmed that the superior yield performance of the ILs was due to the presence of *O. rufipogon* DNA across the target QTL regions.

In the case of *yld9.1*, sib-lines from families 9 and 16 provided an interesting set of contrasts. For family 16, the QTL-containing ILs yielded better than controls, while for family 9, the reverse was true (note: there were no controls for family 13, the highest performing ILs containing *yld9.1*) (Fig. 2). Close inspection of these lines confirmed that the poor performance of lines carrying *yld9.1* in family 9 (IL 9_2-9 and 9_2-10) was not indicative of the value of the *yld9.1* QTL. ILs 9_2-9 and 9_2-10 were both very tall (average 127 cm) and had relatively high lodging (average 10 %), compared to the controls (average 111 cm height and 4 % lodging) while lines from family 16 with *yld9.1* (IL 16_1-1, 16_1-2 and 16_1-10) had an average height of 89 cm and 3 % lodging. The excessive height and lodging of ILs carrying *yld9.1* in family 9 accounted for its poor yield performance in the field. Based on genotypic information, IL 9_2-9 differs from the control lines in family 9, because it not only carries the *yld9.1* introgression but also carries an additional 23 Mb of *O. rufipogon* DNA across chromosome 5, which warrants further study to determine whether it contributes to yield depression or lodging susceptibility.

Evaluation under alternate wetting and drying (AWD) conditions (2008)

To explore the phenotypic plasticity of the interspecific ILs, we estimated genotype × environment (G × E) interaction effects over years and locations under flooded conditions, and also evaluated the ILs under both flooded and AWD conditions during 2008 in Stuttgart (AR) and Beaumont (TX). Under flooded

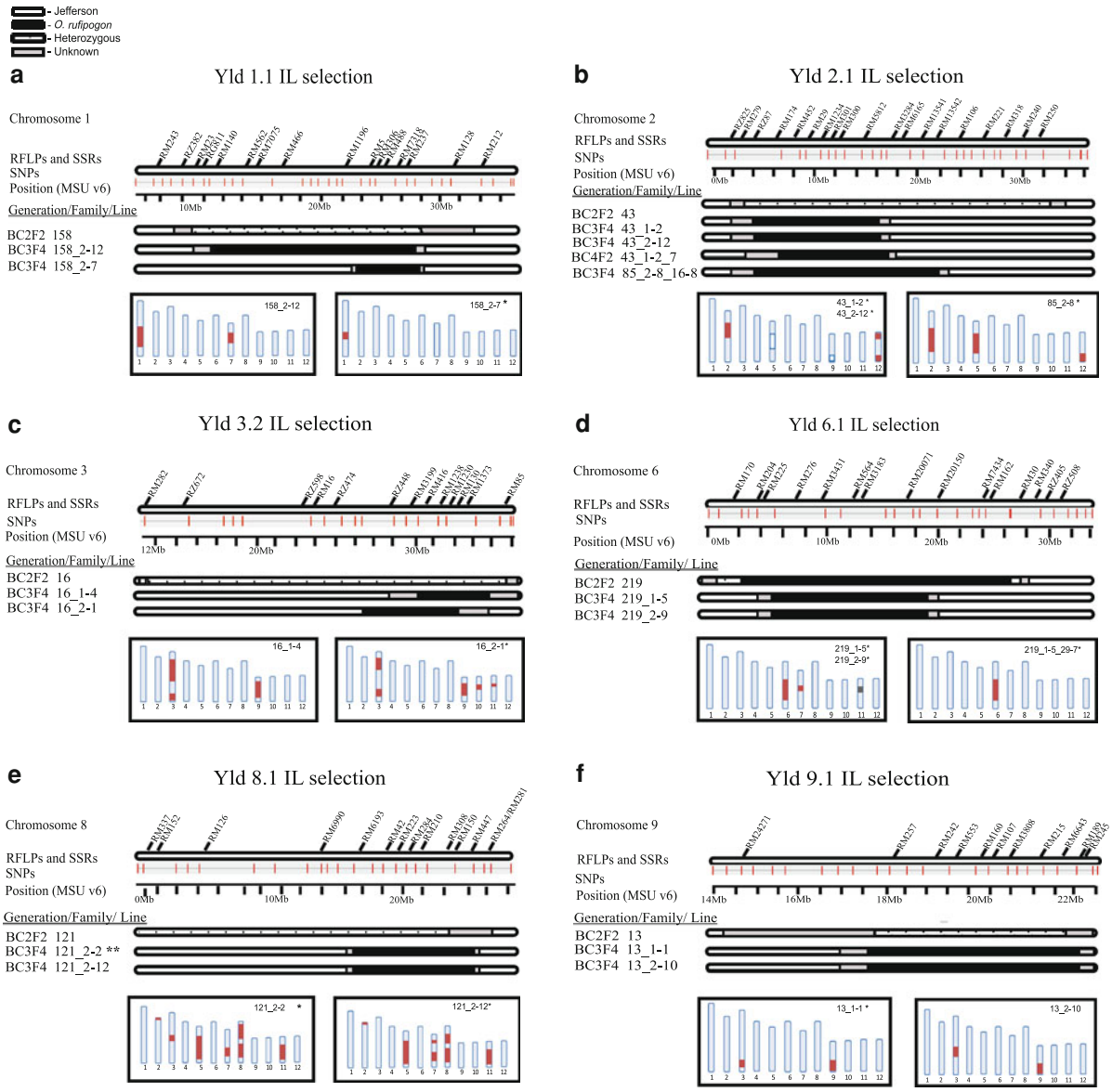

Fig. 1 Graphical genotypes of IL families showing six QTL target regions **a** *yld1.1*, **b** *yld2.1*, **c** *yld3.2*, **d** *yld6.1*, **e** *yld8.1*, **f** *yld9.1*; and background introgressions identified by SNPs, SSRs and restriction fragment length polymorphisms (RFLPs). The *top graph* illustrates the target QTL region marked by SSR and RFLP markers as originally mapped by Thomson et al. (2003); the *row in light gray* indicates 384-SNP assay; *horizontal* chromosome *bars* indicate regions of heterozygosity (*white*), homozygous *O. rufipogon* introgressions (*black*) and Jefferson DNA (*light gray*); *bottom graphs* contain *vertical* chromosomes with regions of introgression indicated in *dark red*; *asterisks* indicate lines selected for submission to GSOR

conditions, we observed significant genotype-by-location and genotype-by-year effects. ILs grown in Stuttgart out-yielded the ILs grown in Beaumont during both 2007 and 2008 ($p < 0.0001$) and the average performance of ILs grown in all locations during 2008 was cumulatively higher than in 2007 ($p < 0.0001$). When plants were grown in AWD conditions they yielded an average of 43.9 % less than the entries in flooded conditions (30.6 % less in Stuttgart and 64.8 % in Beaumont; $p < 0.0001$). The hybrid XL723 yielded significantly better than any of the inbreds under both irrigated and AWD conditions.

Family 43 (*yld2.1*) performed better than Jefferson and other ILs under both irrigated and AWD

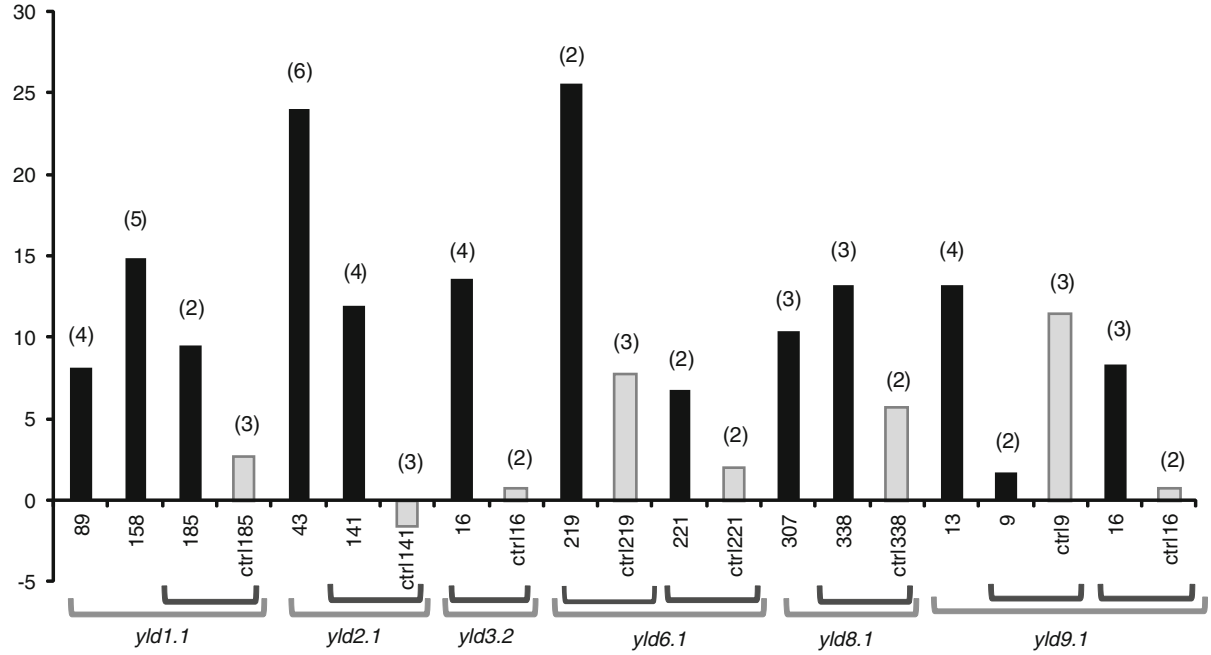

Fig. 2 Percent difference in yield (kg ha^{-1}) relative to Jefferson of BC3 families containing (*black bars*) or lacking (*gray bars*) the *O. rufipogon* introgression at the target QTL evaluated at four flooded locations during 2007–2008. Multiple families containing each QTL are represented by the *lower bracket*, and pairs of families with (*black*) or without (*gray*) the target QTL are indicated by the *upper bracket*. The *number in parentheses above the bars* shows the number of lines in each of the families

conditions, and performed as well as the commercial inbred varieties, Trenasse and Cocodrie ($p = 0.1044$) (Supplemental Fig. 2). Our data suggests that several of the introgressions from *O. rufipogon* mitigate the effect of AWD on grain yield in the Jefferson background, conferring enhanced yield potential under both well-watered and AWD conditions. It is of interest to determine whether these introgressions may also have potential for enhancing yield under both well-watered and AWD conditions in other genetic backgrounds.

Regression analysis was used to identify the traits that were most strongly correlated with yield among the 50 ILs and 20 controls in this study. Eight traits explained 38 % of the variability for grain yield under irrigated conditions (Supplemental Table 4A). The first five traits—panicle length, plant weight (above ground dry biomass), apparent amylose content, seed weight and grain length—explained 35 % of the variation. This along with the correlation analysis indicated that increased yield was associated with longer panicles, heavier, shorter seed and lower amylose content (Supplemental Table 5A). Under AWD conditions, regression analysis identified six parameters that explained >58 % of the variation for yield, and four of them (seed weight, panicle weight, plant height and tiller number) accounted for the majority of the variation (Supplemental Table 4B). Under AWD conditions, fewer traits were significantly correlated with each other compared to irrigated conditions (Supplemental Table 5B).

Stepwise regression analysis was also used to compare the groups of BC3 families and their controls (Table 4) (the same as represented in Fig. 2) to determine the variables which best explained yield. Across these 19 families and Jefferson, model R^2 values ranged from 0.61 to 0.85 and utilized 2–11 variables. However, much less of the variation in yield was explained by the measured traits for *yld2.1* family 43 ($R^2 = 0.32$) and *yld6.1* family 221 ($R^2 = 0.24$). The variables most frequently included in the models were TotalMY, PLTHT, KSDWT, ASV and CHKPCT. For 13 of the 20 BC3 regression models, the primary factor (first variable entered into the

Table 4 Stepwise regression analysis comparing BC3 family groups for variables which explain field yield

QTL	BC3 family	Variable entered in stepwise regression											Proportion of total model R^2 explained by first variable
		1	2	3	4	5	6	7	8	9	10	11	
yld1.1	**158**	**TotalMY**	**PLTHT**	**AVPLTWT**	**AMYLOSE**	**GW**	**KSDWT**	**AVPANWT**	**WholeMY**	**AVTILL**			
	Partial R^2	0.5628	0.127	0.0362	0.0187	0.0351	0.0113	0.0157	0.0096	0.0056			
	Model R^2	0.5628	0.6898	0.726	0.7447	0.7798	0.7911	0.8068	0.8164	0.822			0.78
	185	**KSDWT**	**GL**	**ASV**									
	Partial R^2	0.5389	0.1307	0.0284									
	Model R^2	0.5389	0.6696	0.698									0.77
	89	**AVPLTWT**	**TotalMY**	**GW**	**CHKPCT**	**WholeMY**	**ASV**	**PLTHT**					
	Partial R^2	0.5003	0.0977	0.0862	0.0202	0.0228	0.016	0.0102					
	Model R^2	0.5003	0.598	0.6842	0.7044	0.7272	0.7432	0.7534					0.66
yld1.1 control	**ctrl 185**	**AVPLTWT**	**WholeMY**	**AVSDPAN**	**AVTILL**								
	Partial R^2	0.4138	0.1308	0.0381	0.0762								
	Model R^2	0.4138	0.5446	0.5827	0.6589								0.63
yld2.1	**141**	**PLTHT**	**D2HD**	**TotalMY**	**AVPANWT**	**AVPLTWT**	**AMYLOSE**	**AVPANL**	**Lodge**	**GW**			
	Partial R^2	0.445	0.1418	0.0465	0.0235	0.0303	0.0178	0.0107	0.0117	0.0096			
	Model R^2	0.445	0.5868	0.6333	0.6568	0.6871	0.7049	0.7156	0.7273	0.7369			0.60
	43	**TotalMY**	**AMYLOSE**	**AVPANWT**	**PLTHT**	**GW**	**AVPLTWT**	**GL**					
	Partial R^2	0.0952	0.0551	0.0601	0.035	0.028	0.0221	0.0207					
	Model R^2	0.0952	0.1503	0.2104	0.2454	0.2734	0.2955	0.3162					0.30
yld2.1 control	**ctrl 141**	**PLTHT**	**CHKPCT**	**D2HD**	**ASV**	**AVTILL**	**GL**	**KSDWT**					
	Partial R^2	0.335	0.1759	0.1237	0.0285	0.0598	0.0197	0.0164					
	Model R^2	0.335	0.5109	0.6346	0.6631	0.7229	0.7426	0.759					0.44
yld3.2	**16**	**AVPANL**	**AVSDPAN**	**PLTHT**	**GW**	**WholeMY**	**KSDWT**	**TOTALMY**	**AVPANWT**				
	Partial R^2	0.353	0.0895	0.063	0.0588	0.0258	0.0386	0.0272	0.0339				
	Model R^2	0.353	0.4425	0.5055	0.5643	0.5901	0.6287	0.6559	0.6898				0.51
yld3.2 control	**ctrl 16**	**PLTHT**	**CHKPCT**	**GL**	**ASV**								
	Partial R^2	0.5932	0.0837	0.0684	0.0189								
	Model R^2	0.5932	0.6769	0.7453	0.7642								0.78
yld6.1	**219**	**TotalMY**	**KSDWT**	**AMYLOSE**	**ASV**	**GL**	**D2HD**	**CHKPCT**	**Lodge**				
	Partial R^2	0.3456	0.0491	0.0581	0.0496	0.0615	0.0996	0.0907	0.0274				
	Model R^2	0.3456	0.3947	0.4528	0.5024	0.5639	0.6635	0.7542	0.7816				0.44
yld6.1 control	**ctrl 219**	**KSDWT**	**GW**	**ASV**	**D2HD**	**WholeMY**	**AVPANL**						
	Partial R^2	0.4069	0.0821	0.0627	0.0426	0.067	0.0168						

Table 4 continued

QTL	BC3 family	Variable entered in stepwise regression											Proportion of total model R^2 explained by first variable
		1	2	3	4	5	6	7	8	9	10	11	
	Model R^2	0.4069	0.489	0.5517	0.5943	0.6613	0.6781						0.60
yld6.1	**221**	**GL**	**TotalMY**										
	Partial R^2	0.1268	0.1109										
	Model R^2	0.1268	0.2377										0.53
yld6.1 control	**ctrl 221**	**KSDWT**	**ASV**	**TotalMY**	**D2HD**	**WholeMY**							
	Partial R^2	0.5525	0.1396	0.0632	0.0401	0.0357							
	Model R^2	0.5525	0.6921	0.7553	0.7954	0.8311							0.66
yld8.1	**338**	**D2HD**	**AMYLOSE**	**AVPANL**	**AVSDPAN**	**GW**	**WholeMY**	**CHKPCT**	**Lodge**	**KSDWT**	**GL**	**AVTILL**	
	Partial R^2	0.4562	0.1461	0.0773	0.0486	0.0162	0.0124	0.0188	0.0189	0.0146	0.0124	0.0215	
	Model R^2	0.4562	0.6023	0.6796	0.7282	0.7444	0.7568	0.7756	0.7945	0.8091	0.8215	0.843	0.56
yld8.1 control	**ctrl 338**	**AVPANL**	**D2HD**	**CHKPCT**									
	Partial R^2	0.341	0.0766	0.0348									
	Model R^2	0.341	0.4176	0.4524									0.75
yld9.1	**13**	**PLTHT**	**CHKPCT**	**TotalMY**	**KSDWT**	**AMYLOSE**	**GL**						
	Partial R^2	0.4431	0.0854	0.0318	0.012	0.0192	0.0182						
	Model R^2	0.4431	0.5285	0.5603	0.5723	0.5915	0.6097						0.73
	16	**PLTHT**	**TotalMY**	**AVTILL**	**AVPANWT**	**CHKPCT**	**ASV**						
	Partial R^2	0.4697	0.0859	0.0382	0.0277	0.0372	0.0331						
	Model R^2	0.4697	0.5556	0.5938	0.6215	0.6587	0.6918						0.68
	9	**KSDWT**	**CHKPCT**	**AVPLTWT**	**WholeMY**	**AVTILL**	**GW**						
	Partial R^2	0.359	0.2068	0.0574	0.0563	0.0312	0.0225						
	Model R^2	0.359	0.5658	0.6232	0.6795	0.7107	0.7332						0.49
yld9.1 control	**ctrl 9**	**AVPANL**	**GL**	**AMYLOSE**	**ASV**	**D2HD**	**CHKPCT**						
	Partial R^2	0.3858	0.09	0.0584	0.0412	0.0351	0.0206						
	Model R^2	0.3858	0.4758	0.5342	0.5754	0.6105	0.6311						0.61
Jefferson	**Jefferson**	**GL**	**AVPANWT**	**PLTHT**	**D2HD**	**AVTILL**	**AMYLOSE**	**ASV**					
	Partial R^2	0.1295	0.1039	0.1534	0.0862	0.0158	0.0153	0.0066					
	Model R^2	0.1295	0.2334	0.3868	0.473	0.4888	0.5041	0.5107					0.25

Table 5 Yield and milling yield of yld2_A and yld2_B measured in six locations at the Uniform Regional Rice Nursery (URRN) trials during 2009, compared with three commercial varieties, Presidio, Cocodrie and Trenasse

Line	Yield	StdErr	Grouping	TotalMY	StdErr	Grouping	WholeMY	StdErr	Grouping
IL yld2_A	10,108.2	282.1	A	74.6	0.4	A	49.0	1.9	B
COCODRIE	9,523.4	264.6	AB	72.8	0.2	B	61.2	1.5	A
TRENASSE	9,235.3	279.0	AB	69.8	0.3	D	66.3	1.8	A
IL yld6_A	8,999.1	271.1	B	71.9	0.2	C	60.2	1.0	A
PRESIDIO	8,562.3	221.5	B	71.6	0.2	C	60.4	1.0	A

Yield least squares mean of grain yield, *StdErr* standard error, *Grouping* letter groupings that demarcate the statistical differences between the line as determined by Tukey–Kramer test, letters that are the same within a grouping are not statistically different, statistical differences are at the 5 % level, *TotalMY* total milling yield, *WholeMY* whole milling yield

model) accounted for over 50 % of the total variance in yield. Of these, the variables KSDWT and PLHT were most frequently the primary factors in the models. In contrast, the traits AVSDPAN, AVPANP, Lodge, PLSQM and STDPCT rarely explained variation in yield among the BC3 families and Jefferson.

Yield evaluation in the Uniform Regional Rice Nursery (URRN) in 2009

The best performing ILs, yld2_A (family 43_1-2) and yld6_A (family 219_2-9), were compared to a set of elite lines from the southern USA public rice breeding programs as part of the URRN during 2009. It is noteworthy that the seeding rate of the yield trials in 2007–2008 had been 45 kg ha^{-1} (corresponding to that recommended for the hybrid cultivar that was used as a check), while the URRN plots were seeded at 125 kg ha^{-1}. When yield performance was averaged across six URRN locations, IL yld2_A ranked above Trenasse, Cocodrie, Presidio and IL yld6_A. Despite its higher yield performance, IL yld2_A had poorer whole-milling yield than yld6_A ($p = 0.00248$) or any of the commercial checks (Table 5). IL yld6_A had similar whole-milling yield to the commercial varieties, Cocodrie and Presidio, though it yielded slightly less than Trenasse ($p = 0.0654$). Amylose content (20–22 %), alkali spreading values (4), and their associated markers (RM190 and *Alk*, respectively) indicated that both ILs had conventional long-grain cooking quality similar to Jefferson.

Differences in performance between the 2007 and 2008 studies and the URRN trials suggest that a lower plant density may favor the yield performance of IL yld2_A, making it of particular interest for introgression into hybrid backgrounds where lower commercial seeding rates are commonly used. Yld2_A had consistently higher yield, higher total milling yield, longer grain, later maturity, lower whole milling yield and lower chalk than yld6_A in 2007–2008. These same trends were observed in the 2009 URRN studies, although chalk and grain dimensions were not evaluated. The 2007–2008 grain dimension data indicated that yld6_A had lower grain length:width ratio than yld2_A (2.76 and 2.92, respectively), which may explain the higher whole milling yield that was observed with yld6_A (Table 5 and Supplemental Table 6). In general, the higher grain yield, lower grain chalk, high total milling yield and greater length:width ratio observed in yld2_A are considered more desirable traits than those observed for yld6_A, while its later maturity and lower whole milling yield would be considered less desirable.

Agronomic traits and grain quality

During 2007–2008, we collected data on 17 agronomic and grain quality traits, in addition to yield. These data provided additional information about the BC3 ILs (Supplemental Table 6). Compared to Jefferson, IL yld2_A (line 43_1-2) showed no significant difference in milling yield, amylose content, alkali spreading value (ASV), chalk, grain morphology (length and width), panicle length, panicle weight, number of seeds per panicle, plant height or heading date (Supplemental Table 6). Interestingly, sib-line IL yld2_B (line 43_2-12) had longer average panicle length ($p = 0.0004$) and higher chalk ($p < 0.0001$) than Jefferson, as well as narrower grains ($p < 0.002$)

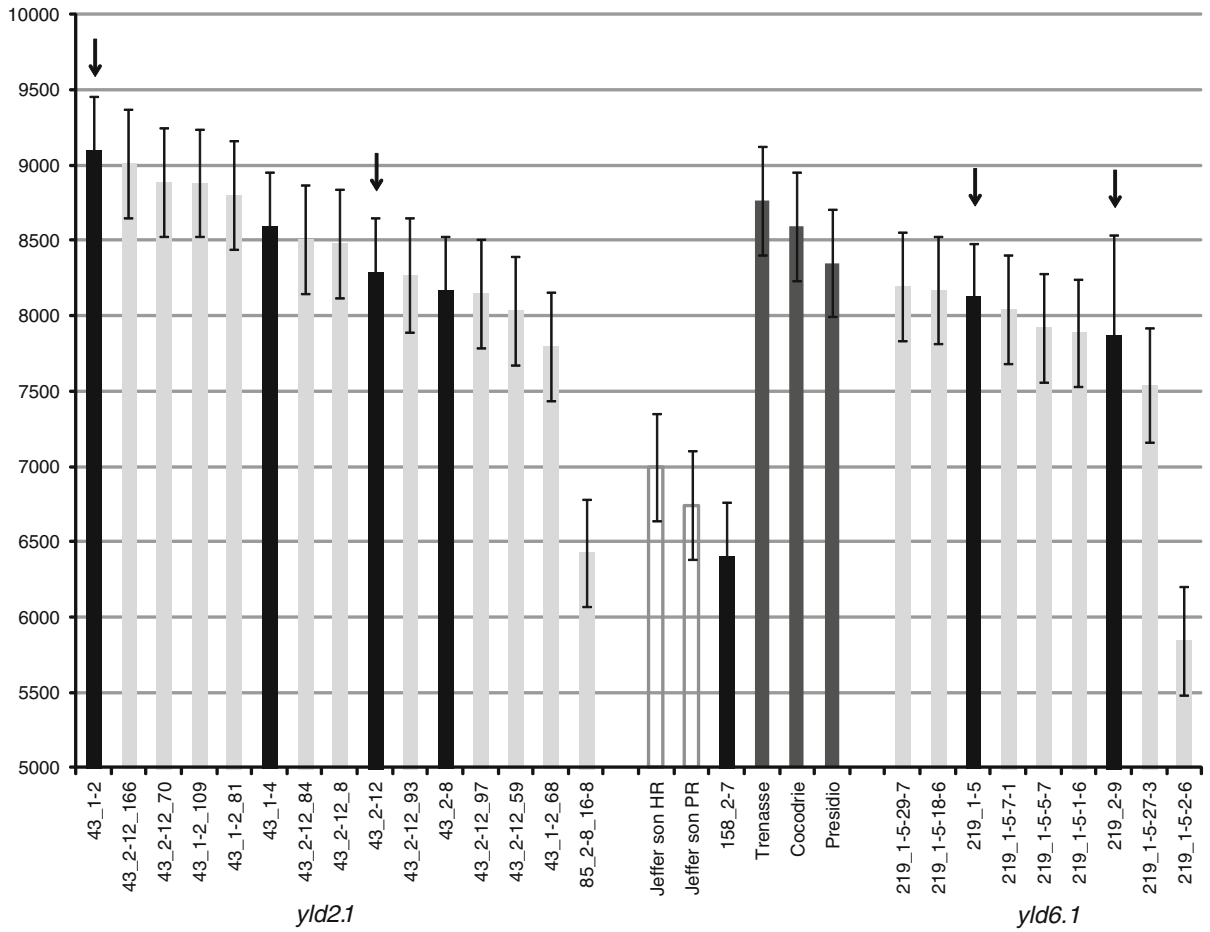

Fig. 3 Mean yield performance (kg ha^{-1}) of *yld2.1* and *yld6.1* BC3 introgression lines and BC4 derivatives in Beaumont during 2010 and both Beaumont and Stuttgart during 2011 field trials. Comparison of the yield performance of ILs (*black bars*) and BC4 derivatives with reduced background introgressions (*light gray bars*) against commercial checks (Trenasse, Cocodrie and Presidio; *highlighted in medium gray*) and Jefferson, the recurrent parent (*white bars*). Jefferson HR is the recurrent parent amplified in Texas in summer 2009 and Jefferson PR is the recurrent parent amplified in the winter nursery in Puerto Rico. BC3 lines submitted to the GSOR are *highlighted with arrows*. MRG5836 (a marker associated with *Pi-z* located at 9.3 Mb) is annotated as a *black arrow* between SNP position id6005761 (8.98 Mb) and id6006235 (10.01 Mb) on the iPLEX MassARRAY track

than yld2_A. IL yld6_A (line 219_2-9) had shorter grain length ($p < 0.0001$), shorter average panicle length ($p = 0.02$), less panicle weight ($p < 0.01$), lower milling yield, lower amylose ($p < 0.0147$), lower alkali spreading value ($p < 0.0123$), and was higher in chalk ($p < 0.0001$) than Jefferson, but it showed no significant difference in plant height or flowering time.

The most serious problem associated with many of the other ILs was significantly higher levels of chalk and small differences in grain morphology and/or grain weight (Supplemental Table 6). Interestingly, none of the selected ILs were significantly different in plant height or flowering time, and panicle characteristics remained relatively stable among the ILs, suggesting that the introgressions from *O. rufipogon* affected basic physiological components of yield performance without dramatically affecting plant architecture or morphology in the Jefferson background.

Homogeneity of IL families

Using whole-genome SNP assays and targeted SNP markers, we measured the level of homozygosity and the homogeneity of ILs that had undergone field-based

seed propagation for 4 years (2007–2010). As summarized in Supplemental Fig. 3, by the BC3F8 generation, IL yld2_A (line 43_1-2) had been fixed for *O. rufipogon* alleles in the *yld2.1* region as well as in background introgressions on chromosomes 5, 9 and 12. On the other hand, for IL yld6_A (family 219_2-9), three out of 46 plants, representing eight different headrows in the BC3F8 generation, were still segregating across the target region on chromosome 6 (Supplemental Fig. 4).

Genotyping assays to facilitate utilization of ILs in breeding

A total of 42 KASP primer sets and three separate MassARRAY iPLEX assays (81 markers) were designed based on polymorphic SNP positions identified by the 44K-SNP array (Zhao et al. 2011). These SNP markers were chosen to facilitate selection for the target *O. rufipogon* introgressions in IL yld2_A and IL yld6_A, and against *O. rufipogon* introgressions elsewhere in the genome. Markers were selected at approximately 0.5-Mb intervals (Supplemental Tables 1 and 2). In addition, locus-specific markers, AP5659-1, AP5659-5 and RM224, were used to confirm the presence of blast resistance alleles inherited from Jefferson at the *Pi-z* and *Pi-km* loci (on chromosomes 6 and 11, respectively) in IL yld2_A (family 43_1-2), and to confirm that IL yld6_A (family 219_2-9) carries resistance only at the *Pi-km* locus. These marker sets (see Supplemental Note on Gene-Specific Markers) provide economical and efficient selection platforms so that breeders can readily utilize these ILs as parents in future plant improvement.

Submission of ILs to the Genetic Stocks *Oryza* Collection (GSOR)

Twelve ILs were deposited in the GSOR Collection (http://www.ars.usda.gov/Main/docs.htm?docid=8318) for use in future genetic analysis and as parents in breeding programs (Table 3). These lines contain homozygous *O. rufipogon* introgressions across each of the six target QTL regions, and have minimal *O. rufipogon* DNA in the genetic background. Of the 12 lines, eight were selected to represent each of the six QTL targets following the BC3 multi-location yield trials, and the remaining lines represent BC4 derivatives from four of the same families.

Genotype data based on whole-genome SNP assays (see Materials and methods) provided information about the size of the introgressed regions and the number of background introgressions in each of the ILs. These data were used to compute the percent recurrent parent in each of the lines based on the number of polymorphic markers out of the total number of markers genotyped (Table 3).

Influence of background introgressions

The 12 ILs are at different stages of backcrossing (BC3–BC4). While some of the lines containing multiple background introgressions out-performed siblings that contained fewer donor fragments, in most cases reducing the number of *O. rufipogon* background introgressions made little difference to the yield performance of the lines (Fig. 3). Thus, in the 2010–2011 yield trials, the performance of BC4 IL derivatives was generally similar to the corresponding BC3 ILs.

A comparison among lines within a family or comparisons among families allowed us to refine the position of the yield QTL or associate other phenotypic characteristics with individual *O. rufipogon* introgressions. For instance, IL yld2_D (IL 85_2-8_16-8) from family 85 carried a target introgression at *yld2.1* that was smaller and slightly offset compared to the introgression across the same region in family 43. This line yielded 11.4 % less than Jefferson during 2010 (Fig. 3) and was observed to be highly susceptible to straighthead under natural soil conditions (data not shown). The fact that IL yld2_D carried two background introgressions on chromosomes 5 and 10 suggests that one or both of these introgressions may be responsible for the straighthead susceptibility (Fig. 1b).

For *yld6.1*, several representatives of family 219 were evaluated. IL yld6_B (family 219_1-5) contained the same target introgression as IL yld6_A, along with two background introgressions, while IL yld6.1_C (219_1-5_29-7) was a backcross derivative of IL yld6_B that contained no detectable background introgressions at all (Fig. 1d). During 2010–2011, IL yld6_B and IL yld6_C both out-yielded Jefferson by more than 17 % (Fig. 3), while a sib-line, 219_1-5-2-6, yielded 16 % less than Jefferson. This sib-line has a recombination breakpoint across the target region and provides material for future fine-mapping purposes.

IL yld1_A (family 158_2-7), IL yld3_A (family 16_2-1), IL yld8_A (family 121_2-2) and IL yld9_A (family 13_1-1) out-performed Jefferson by 15.4–17.5 % in the 2007–2008 trials under a reduced seeding rate (45 kg ha^{-1}) when averaged across four locations and 2 years (Table 3) and, interestingly, IL yld1_A (family 158_2-7) yielded less than Jefferson in the 2010–2011 field trials where the planting density was higher (seeding rate 125 kg ha^{-1}) (Fig. 3). These lines provide an opportunity to study the genetics of how plants perceive and respond to different planting densities in the field.

Discussion

We used backcrossing with MAS to develop ILs carrying six different yield QTL and evaluated them over 2 years in multi-location yield trials in the southern USA. Two ILs carrying introgressions from the wild donor (*O. rufipogon*) across yield QTL on chromosomes 2 and 6 consistently out-performed the recurrent parent, Jefferson (6,217 ± 810 kg ha^{-1}) and were similar in yield to the newer commercial varieties, Trenasse (8,097 ± 812 kg ha^{-1}) and Cocodrie (8,244 ± 811 kg ha^{-1}). While all inbred lines (both ILs and commercial varieties) yielded significantly less than the commercial hybrid, XL723 (10,336 ± 811 kg ha^{-1}), these results suggest that specific introgressions from a low-yielding wild ancestor can significantly enhance performance when introduced into an elite cultivated background. Previous collaboration with the China Hybrid Rice Research Institute (Xiao et al. 1998) resulted in introgression of the *yld2.1* QTL from *O. rufipogon* (IRGC 105491) into elite Chinese hybrid backgrounds, where it has significantly enhanced the yield of super-hybrid rice in China (Wu et al. 2010). This suggests that the introgression(s) identified in this study have potential for improving the yield performance of elite inbreds and hybrids from diverse genetic backgrounds.

Yield QTL in the Jefferson × *O. rufipogon* population were originally identified by Thomson et al. (2003) based on average seed weight per BC2F2 family estimated from a bulk of ten plants grown in small family plots. In the current study, yield performance of the ILs was evaluated using large-scale field trials and BC3F4–F5 progeny. In field trials (2007–2008), yield was measured in terms of grain weight per plot area (kilograms per hectare). Thus, there was a critical difference as to how yield was estimated in the original QTL study by Thomson et al. (2003) (per plant basis) and how yield was assessed in the yield trials during 2007–2011 (per plot basis). It is noteworthy that this work has confirmed the yield-enhancing quality of the same QTL reported by Thomson et al. (2003) regardless of whether it was measured on a per plant or field plot basis.

Chalkiness is an important grain quality trait causing breakage during the milling process and decreases the market value of rice. Certain environmental conditions influence the amount of chalk, and certain grain characteristics are associated with the amount of breakage (as reviewed in Nelson et al. 2011). Line 16_1-6 was developed as a control for *yld3.2* and *yld9.1* (Table 2) and, while it did not yield as well as Jefferson, chalk levels were significantly lower than in Jefferson (adjusted $p < 0.015$) and similar to those of Cocodrie. Apart from a 24.5-Mb introgression downstream from the *yld3.2* target, there is only one 4.5-Mb background introgression on chromosome 11 segregating in the family (data not shown). In the future, IL 16_1-6 will be crossed to the higher yielding ILs in an effort to lower chalk levels and increase grain yield.

High-yielding IL yld2_B (43_2-12) had significantly narrower grain width than IL yld2_A (43_1-2) (Supplemental Table 6), while IL yld2_A was not significantly different from Jefferson. Two genes determining grain morphology in rice, *grain size 5* (*GS5*) and *grain width 5* (*GW5*), reside at 3.45 Mb and 5.35 Mb, respectively, on rice chromosome 5 (Li et al. 2011; Shomura et al. 2008). Both genes fall within the *O. rufipogon* introgression that is present in IL yld2_A, but absent in IL yld2_B, and are implicated as a reason why the *O. rufipogon* introgression across this region of chromosome 5 is associated with increased grain weight, as reported by Thomson et al. (2003). *GW5* was shown to bind with ubiquitin, acting in the ubiquitin–proteasome pathway to regulate cell division during seed development, and a 1.2-kb deletion in the open reading frame region was associated with increased grain width (Shomura et al. 2008; Weng et al. 2008). In our study, neither Jefferson nor *O. rufiopogon* carried the derived allele conferring

increased grain width, so this was ruled out as an explanation for the difference in grain width between the two IL sib lines. *GS5* was shown to positively regulate the plant cell cycle to increase cell number and, to some extent, cell size, leading to enhanced latitudinal growth in the grain (Li et al. 2011). Three promoter haplotypes at *GS5* were predictive of grain width, grain weight and grain filling characteristics in 35 *O. sativa* cultivars (Li et al. 2011). It would be of interest to undertake a higher resolution study to investigate the relationship between the alleles from our *O. rufipogon* donor (IRGC 105491) and their interaction with Jefferson alleles at these and other genes in the grain size pathway to determine whether they contribute to the variation for grain width and/or chalk observed in our material.

Based on the Thomson et al. (2003) study, *yld3.2* explained the greatest proportion of variance for yield ($R^2 = 16$ %) of the six yield-QTL targets evaluated. However, the ILs developed for *yld3.2* did not perform as well as other lines, possibly due to the numerous background introgressions that remained in all the *yld3.2*-containing lines. IL yld3_A (family 16_2-1) contained 11.9 % *O. rufipogon* SNP alleles (78/653). This family carried a 14-Mb introgression on chromosome 9 that contained the QTL target, *yld9.1*. When the performance of IL yld3_A was compared to IL yld9_A (family 13_1-1), which contained a 5-Mb introgression across the target region on chromosome 9 but no introgression on chromosome 3 (and only 2.9 % donor alleles across the entire genome), the two lines yielded similarly (and were not significantly different from Jefferson) across years and locations. While *yld3.2* does not coincide with previously reported yield-related QTL, *yld9.1* co-locates with QTL reported as *TGW9 (thousand grain weight)* detected in a Minghui 63 × Teqing RIL population (Liu et al. 2010) and a QTL cluster including *gw9.1*, *hd9.1* and *ph9.1* detected in a Hwaseongbyeo × *O. rufipogon* BC population (Xie et al. 2008). Further backcrossing to separate the effects of the target QTL regions in various backgrounds will be needed to better understand the potential of *yld3.2* and *yld9.1*.

Several genome-wide SNP assays were used in this study. Levels of polymorphism for the Jefferson × *O. rufipogon* population are provided in Supplemental Table 7 for the 384-SNP Illumina BeadXpress OPA 6.0 (76 %), the 1,536-SNP Golden Gate assay (42.5 %) and the 44K-SNP Affymetrix array (42.6 %). Polymorphic SNPs on the 1,536-SNP assay (Zhao et al. 2010), were not always well distributed across the genome, but we were able to design a 384-SNP "breeder's chip" optimized for *tropical japonica* × *O. rufipogon* populations (OPA6.0) (Thomson et al. 2011) that was both efficient and economical (USD25–35 per sample) for use in marker-assisted backcross conversion on this project. SNPs for the breeder's chip were reliably and efficiently selected from the 44K-SNP dataset published by Zhao et al. (2011).

We also developed targeted SNP assays (KASP and iPLEX MassARRAY) that will aid the swift integration of *O. rufipogon* introgressions into other *tropical japonica* backgrounds. These targeted assays are ideal for backcross conversion where the wild introgressions are selected for either retention or elimination, and where accurate detection of *O. rufipogon* alleles in either the homozygous or heterozygous condition is important. These SNPs are also useful for fine-mapping experiments and more in-depth molecular analysis of this material, though they may not be useful for crosses with *indica* or *aus* varieties. To design informative SNP assays for other types of genetic materials, researchers may consult the rice diversity datasets generated by Zhao et al. (2010, 2011) on 400 *O. sativa* varieties using the 44K-SNP Affymetrix chip or the 1,536-SNP Illumina BeadXpress assay (www.ricediversity.org). Based on experience, approximately 90 % of all SNP markers selected from the pool of polymorphic targets identified on the 44K-SNP Affymetrix chip were directly functional in Illumina, KASP and iPLEX MassARRAY systems (McCouch et al. 2010).

Marker-assisted selection offers great advantages to breeders and geneticists interested in introgressing large effect QTL from one genetic background to another. An important lesson from this study is that using a combination of MAS to retain a target QTL and phenotypic selection for the best performing lines within a family carrying that QTL often results in the retention of favorable background introgressions from the donor parent. These background introgressions, identifiable by whole-genome marker assays, appear to confer favorable genotype-by-genotype interactions (G × G) in the selected ILs. G × G effects are known to occur among independent *O. rufipogon* introgressions in ILs, as well as

between specific *O. rufipogon* introgressions and loci in the elite, recurrent parent genetic background (Maas et al. 2010). Use of whole-genome SNP assays can quickly identify non-target regions of genetic polymorphism likely to account for differences in performance among family members. This study demonstrates the power of marker-assisted backcrossing to capture yield-enhancing transgressive variation from a low-yielding wild ancestor in the genetic background of an elite cultivar, and confirms that QTL identified in the BC2 generation on an individual plant basis can be a predictor of enhanced productivity in large-scale yield trials conducted over years and environments.

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